

*Transferred manuscripts:*

*Editorial Note: this manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at Nature Communications.*

NCOMMS-16-12317-T

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have substantially improved the manuscript to address all comments I raised in the previous review. I am happy to support publication without further changes.

The focus on the single improved origami design, and the voltage-dependence of translocation speed helps satisfy my initial concerns that this pore was functioning 'as designed'. In particular highlighting the significant magnitude of the current blockades relative to open-pore currents helps emphasize the advance.

My only suggestion would be to include a comment to the effect that they attempted PEG-sizing of the pore but were unsuccessful.

Reviewer #2 (Remarks to the Author):

I am reviewer #2 for the first version of this manuscript (submitted to Nature Nanotechnology). I continue to hold my view that this work is novel (structurally rigid DNA nanopore, detergent-free insertion to GUVs, and DNA translocation), thorough (well documented statistics and analyses), and potentially impactful (applications in cell-size reactor). All my previous concerns have been adequately addressed. Overall, I feel this revised manuscript is ready to publish in Nature Communications. I have only one request: could the authors PLEASE include cross-section views with helix numbers together with the caDNAno designs in SI for the benefit of non-specialists?

Reviewer #3 (Remarks to the Author):

The manuscript by Simmel et al. is a resubmission of a revised manuscript. After reading the comments, responses and manuscript, I am convinced that the manuscript is suitable for publication. The responses are clearly laid out and address the concerns of the referees.

As a note, the authors describe in the manuscript that other mechanisms of additional current rather than through the pore cannot be ruled out. In this respect, the authors need to discuss a recent paper by Stulz, Aksimentiev and Keyser (Nano Lett 2016) and add some comments on the findings on current formation alongside simple DNA pores; this is important in view of this paper's mentioning that leakage along the sidewalls of the pore structure could be significant.

## **Reply to the reviewer's comments:**

We thank all reviewers for their very positive assessment of our revised manuscript.

\* Reviewer #1

Comment:

The authors have substantially improved the manuscript to address all comments I raised in the previous review. I am happy to support publication without further changes.

The focus on the single improved origami design, and the voltage-dependence of translocation speed helps satisfy my initial concerns that this pore was functioning 'as designed'. In particular highlighting the significant magnitude of the current blockades relative to open-pore currents helps emphasize the advance.

My only suggestion would be to include a comment to the effect that they attempted PEG-sizing of the pore but were unsuccessful.

Reply:

We included the suggestion adding a comment on unsuccessful PEG-sizing in the Supplementary Information (page 26)

\* Reviewer #2

Comment:

I am reviewer #2 for the first version of this manuscript (submitted to Nature Nanotechnology). I continue to hold my view that this work is novel (structurally rigid DNA nanopore, detergent-free insertion to GUVs, and DNA translocation), thorough (well documented statistics and analyses), and potentially impactful (applications in cell-size reactor). All my previous concerns have been adequately addressed. Overall, I feel this revised manuscript is ready to publish in Nature Communications. I have only one request: could the authors PLEASE include cross-section views with helix numbers together with the caDNAo designs in SI for the benefit of non-specialists?

Reply:

We included the cross-section views as requested for the three origami pore designs in the Supplementary Information (page 19, 20, 21)

\* Reviewer #3

Comment:

The manuscript by Simmel et al. is a resubmission of a revised manuscript. After reading the comments, responses and manuscript, I am convinced that the manuscript is suitable for publication. The responses are clearly laid out and address the concerns of the referees.

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Reply:

We cite the paper and discuss it in the Main text (page 5) and Supplementary Information (page 29).